



Kongresi i simpoziji (2022 – 2024):

Prilog sa skupa (u zborniku)

Kratko priopćenje

1. Batistić, Mirna; Garić, Rade; Hure, Marijana; Pasković, Nika; Ursella, Laura; Cardin, Vanessa; Civitarese, Giuseppe; Gačić, Miroslav; Misericchi, Stefano
Vertical distribution of net zooplankton biomass at the time of winter vertical mixing in the open southern Adriatic Sea (Mediterranean Sea) // EGU24 / EGU (ur.).
Beč: EGU General Assembly 2024, 2024, 10533, 1

The open southern Adriatic Sea with a maximum depth of 1242 m is one of the three sites of open deep-sea convection in the Mediterranean Sea. The effects of winter vertical mixing on zooplankton biomass were investigated in the open southern Adriatic Sea in February 2023. Samples were collected using Nansen nets (250 μm mesh size) in eight layers from 0 to 1200 m depth during day and night. The highest biomass values were sampled in the deeper layers below 300 m depth (twice as high as in the upper layers) both in the day and night samples. This could be related to vertical mixing in several pathways. This event was triggered by cold winter conditions and significant heat loss in the previous days, which together with the inflow of high salinity water from the eastern Mediterranean (38.96) caused strong vertical mixing down to 600 m depth. As a result of this event, relatively high chlorophyll-a concentrations (max. 0.33 mgm^{-3}) were measured down to 600 m depth. Therefore, due to the vertical mixing, deeper layers received more food than usual from the surface, so that more food was available for deep-sea zooplankton organisms and they did not have to migrate upwards. The effect of vertical mixing in winter was also clearly visible in some zooplankton organisms that cannot effectively resist the vertical currents, so that they also contribute to the increase in biomass at depth. This is confirmed by the backscattering strength (Sv) data, which show that convective mixing resulted in a smeared Sv signal, indicating that the plankton was transported to deeper layers and no migration took place. Future studies should consider the influence of open-sea convective events on vertical carbon export in the oligotrophic southern Adriatic.

2. Pasković, Nika
First record of the dinoflagellate *Tripos rotundatus* in the Adriatic Sea // 7th Faculty of Science PhD Student Symposium - Book of Abstracts / Pavlek, Katarina (ur.).
Zagreb: Prirodoslovno-matematički fakultet Sveučilišta u Zagrebu, 2023. str. 135-135

Dinoflagellates are an important group of protists in marine and freshwaters with a remarkable diversity of life forms (free-living, parasites and mutualistic symbionts), habitats (plankton and benthos) and nutrition modes (heterotrophic, chloroplast-containing) [1]. About ~6000 taxa (species and infraspecific units) of dinoflagellates are currently accepted [2]. In the Mediterranean Sea, 673 taxa have been identified, while in the Adriatic 322 taxa have been reported [3]. The genus *Tripos* Bory is globally widespread in marine waters and is the most diverse genus among dinoflagellates with ~800 taxa described. These species are typically large and robust, often with horns [4]. Members of this genus were previously included in the genus *Ceratium* Schrank until morphological and molecular data supported restricting *Ceratium* to freshwater species [5]. According to my best knowledge, the taxa *Tripos rotundatus* (Jørgensen) F.Gómez represents the first record for the Adriatic and the Mediterranean Sea [6]. The taxa were found in a plankton net sample originating from the coastal station in the southern Adriatic, in the summer of 2021. A morphologically very similar species is *Tripos digitatus* (Schütt) Gómez [6], which was found in the Mediterranean [7,8] and in the northern Adriatic as well [9]. The morphological characteristic between these two taxa has been recently explained [5]. Their epitheca and hypotheca are distinct, with the epitheca of *T. digitatus* is strongly reflected towards the dorsal side, the left antapical horn is anteriorly directed and the apex showed a short projection. In *T. rotundatus* epitheca is less bent towards the dorsal side, the short projection on the apex is missing, and the left antapical horn is directed laterally. This finding contributes to a better understanding of the diversity of dinoflagellates in the Adriatic Sea.

Sažetak izlaganja sa skupa

3. Pasković, Nika; Dupčić Radić, Iris
Planktonic diatoms in the open southern Adriatic Sea in 2022 // Book of abstracts of 15th European Diatom Meeting / Levkov, Zlatko; Mitić Kopanja, Danijela; Zaova, Dušica (ur.).
Skoplje: Faculty of Natural Sciences and Mathematics, Skopje, 2024. str. 184-185

The Adriatic Sea, situated as a semi-enclosed basin within the eastern Mediterranean, with its deepest part in the southern Adriatic, reaching depths of approximately 1250 meters in the Southern Adriatic Pit (SAP). Characterized as oligotrophic, the southern Adriatic is primarily dominated by nanophytoplankton and picophytoplankton. Nevertheless, higher microphytoplankton (cells >20 µm) abundances (>10⁵ cells L⁻¹) are a recurrent phenomenon, especially in early spring. In 2022, comprehensive seasonal studies were conducted on the hydrography, nutrient concentrations, biomass, structure and dynamics of the phytoplankton community in the SAP. Four sampling cruises were carried out, during which 41 phytoplankton samples were collected. Phytoplankton abundances were estimated using the Utermöhl method. Nanophytoplankton abundances prevail the microphytoplankton by an order of magnitude in all seasons. The highest nanophytoplankton (1.37×10⁶ cells L⁻¹) and microphytoplankton (3.3×10⁵ cells L⁻¹) abundances were found in the spring, coinciding with the highest observed chlorophyll a concentrations. A total of 142 taxa (species and intraspecific taxa) were recorded: 77 dinoflagellates, 55 diatoms, 9 coccolithophores, and one silicoflagellate. The diatom genera with the greatest number of taxa were: *Chaetoceros*, *Thalassionema*, *Navicula*, *Leptocylindrus*, and *Pseudo-nitzschia*. The nutrient concentrations were characteristic of the oligotrophic conditions in southern Adriatic. Maximum

concentrations of phosphate and silicate were noted in autumn, and total inorganic nitrogen reached its maximum in summer. Vertical water column mixing occurred during winter, creating conditions suitable for phytoplankton development. In 2022, the phytoplankton abundances were dominated by nanophytoplankton (2–10 µm) in all seasons, while diatoms dominated the microphytoplankton in winter-spring period (80.797.1%). Dinoflagellates prevail during summer (76.9%) and autumn (54.4%). The primary production and diversity of phytoplankton in the southern Adriatic are influenced by hydro-meteorological conditions and the inflow of different water masses. The circulation of the Bi-modal Oscillating Mechanism (BiOS) between the Adriatic Sea and Ionian Sea determines the water mass entering the southern Adriatic.

4. Jasprica, Nenad; Dupčić Radić, Iris; Pasković, Nika
Diatom community during vertical mixing in the open Southern Adriatic Sea, NE Mediterranean // Book of abstracts of 15th European Diatom Meeting / Levkov, Zlatko; Mitić Kopanja, Danijela; Zaova, Dušica (ur.).
Skoplje: Faculty of Natural Sciences and Mathematics, Skopje, 2024. str. 180-181

Deep water convection in winter is one of the major processes driving primary productivity in open waters, such as in the Southern Adriatic Sea (eastern Mediterranean Sea). This process is highly variable in time, depending on the specific oceanographic and meteorological conditions (circulation, stratification, sea-atmosphere interactions) of each specific winter. Phytoplankton community can be a good indicator of occurrence of such convective events. The high abundances and biomass (chlorophyll a, Chl-a) of phytoplankton in the open Southern Adriatic Sea observed in March 2017 are the result of convection-driven nutrient enrichment in the upper layer, followed by a period of water column stability that promotes phytoplankton development. The diatom bloom commenced in early March 2017, reaching its peak approximately two weeks later, as also evident from satellite-derived chlorophyll-a (Chl-a) data. It occurred during the greatest SiO₄, PO₄ and NH₄ availability. Diatoms had the highest contribution to the phytoplankton abundance (21.1-41.0%) from 3rd to 20th March 2017, with values from 6.1×10^2 to 5×10^4 cells L⁻¹ and 2×10^2 to 1.9×10^5 cells L⁻¹ on 3rd and 20th March, respectively. During the peak (20th March) diatoms were mostly composed of *Pseudo-nitzschia* spp. (19.8%), *Chaetoceros* spp. (13.4%), *Ch. lorenzianus* (12.0%), *Ch. affinis* (11.1%) and *Asterionellopsis glacialis* (9.6%). The majority of the diatom population occurred in the surface layer (0–50 m). Subsequently, four days later (24th March), the contribution of diatoms markedly decreased (to 1%), while that of coccolithophorids slightly increased (to 2–3%). In general, the bloom-type diatom community (*Chaetoceros*, *Pseudo-nitzschia*, *Nitzschia*, etc.) suggests that a high nutrient environment supported the growth of species typical for the summer bloom of the southern Adriatic coastal waters, i.e. in areas of higher trophic levels and influenced by anthropogenic nutrient loads. These species are generally the main contributors to high Chl-a in cases of intense physical dynamics, i.e., during winter convection. Some species (e.g., *Skeletonema*) may originate from the northern Adriatic source area, and reach the deep SAP either by southward currents along the western Adriatic coast and/or by crosswise flow within the cyclonic gyre and mesoscale eddies from the eastern or western Adriatic coast.

5. Vodopivec, Martin; France, Janja; Jasprica, Nenad; Pasković, Nika; Batistić, Mirna; Mozetič, Patricija

Changes in the timing of phytoplankton blooms: comparison between northern and southern Adriatic // EGU24 / EGU (ur.).

Beč: EGU General Assembly 2024, 2024, EGU24-7817, 7817. doi: 10.5194/egusphere-egu24-7817

The phenology of phytoplankton blooms holds significant implications for marine ecosystems as it shapes pelagic food webs. The onset, intensity, and duration of phytoplankton blooms, along with their synchronization with zooplankton cycles, can impact the survival rates of these species and overall community production. In this study, we employ a combination of in situ and satellite-derived chlorophyll concentrations, utilizing various statistical methods to discern the presence and timing of spring and autumn blooms in different regions of the Adriatic Sea. The northern Adriatic (NA) represents a coastal, river-dominated ecosystem influenced by anthropogenic nutrient enrichment, with a recent decline observed in chlorophyll concentration and primary production. Conversely, the southern Adriatic (SA) is characterized as a true pelagic ecosystem with minimal influence from coastal waters on nutrient levels. Here, primary production is primarily controlled by meteorological conditions that dictate convective mixing and nutrient availability for autotrophic uptake. Our analysis reveals that the northern Adriatic predominantly experiences both spring and autumn blooms, whereas the southern Adriatic witnesses only autumn blooms, peaking in late autumn or winter. We investigate trends in the timing of the onset and peak of phytoplankton blooms, searching for environmental factors influencing these shifts. As anticipated, the onset of the autumn bloom is found to be delayed, with statistically significant trends observed in specific areas. It is worth noting that the lack of statistical significance in some instances may be attributed, at least in part, to the relatively short period of available satellite data (from 1997 onwards).

6. Batistić, Mirna; Garić, Rade; Hure, Marijana

Occurrence of warm-water affinity gelatinous zooplankton species in the Adriatic Sea in the last three decades // 5. hrvatski simpozij o invazivnim vrstama : zbornik sažetaka / 5th Croatian Symposium on Invasive Species : book of abstracts / Jelaska, Sven (ur.).

Zagreb: Hrvatsko ekološko društvo, 2023. str. 64-64

Analysis of plankton samples from the southern Adriatic from 1993 to 2022 revealed significant changes in the community of gelatinous zooplankton. Sixteen species were detected in the Adriatic Sea for the first time, while three species reappeared after years of absence. Of these newly recorded species, eleven were thermophilic, and were recorded during the periods from 2001 to 2007 and from 2013 to 2022. Their occurrence is directly related to the strong influx of water from the eastern Mediterranean into the Adriatic and to the fact that the warming of the Adriatic has accelerated, especially in recent decades. Of the thermophilic species recorded, *Ferosagitta galerita*, *Lensia subtiloides* and *Paracytaeis octona* originate from the Indo-Pacific and most likely entered the eastern Mediterranean as Lessepsian migrants. The same pathway of introduction probably applies to other eight recorded warm-water species with circumglobal distribution. In general, warming in the Adriatic Sea, especially in the last decade, favours the establishment of populations of tropical, potentially invasive, zooplankton species. We therefore emphasise that monitoring activities that include less well-studied organism groups such as gelatinous zooplankton are important to establish baselines for the detection of non-indigenous species at an early stage of their colonisation.

7. Garić, Rade ; Batistić, Mirna ; Hure, Marijana ; Baričević, Ana ; Smodlaka Tanković, Mirta ; Dénes, Marcell
Application of metabarcoding in detecting non-indigenous zooplankton species in the Adriatic // 5. hrvatski simpozij o invazivnim vrstama : zbornik sažetaka / 5th Croatian Symposium on Invasive Species : book of abstracts / Jelaska, Sven (ur.).
Zagreb: Hrvatsko ekološko društvo, 2023. str. 66-66

We used the COI metabarcoding approach to assess it as a tool for detecting non-indigenous gelatinous zooplankton species entering the Adriatic. Duplicate samples were collected at a coastal Lokrum station (100 m deep) from the bottom to the surface using a Nansen zooplankton net with a 200 µm mesh size. Samplings were conducted monthly from March 2021 to February 2022. One of the samples was preserved in formalin for species abundance and composition analysis under binocular, while the other was preserved in ethanol for DNA extraction and metabarcoding. Metabarcoding was performed using mICOLintF-jgHCO2198 primer pair, which amplified a part of the COI gene about 300 bp in length. Out of five non-indigenous species detected by microscopy, only one was recorded in metabarcoding data. On the other hand, the metabarcoding approach detected two new gelatinous zooplankton species for the Mediterranean, which couldn't be verified by microscopy. Our results suggest that metabarcoding can detect non-indigenous species, but it is not a reliable tool if it is not tuned to specific species. Detection relies on both the abundance of the target species and the level of identity between the primers and the COI sequence of the target species.

8. Hure, Marijana ; Garić, Rade ; Baričević, Ana ; Smodlaka Tanković, Mirta
Application of metabarcoding in detecting non-indigenous copepod *Pseudodiaptomus marinus* // 5. hrvatski simpozij o invazivnim vrstama : zbornik sažetaka / 5th Croatian Symposium on Invasive Species : book of abstracts / Jelaska, Sven (ur.).
Zagreb: Hrvatsko ekološko društvo, 2023. str. 65-65

The free-living pelagic copepod *Pseudodiaptomus marinus* Sato, 1913, native to the northwestern Pacific Ocean, is the species that is probably spreading most rapidly in European and neighbouring waters. The primary vector of introduction is ballast water, while secondary dispersal by local ship traffic and coastal circulation could promote further spread. To test metabarcoding as a tool for detecting *P. marinus* and to determine its abundance levels, we seasonally sampled various coastal areas, such as the Mljet Lakes National Park, protected area of Mali Ston Bay, the protected Lake Mir on the island of Dugi otok, the Pag channel and Novigrad Sea. Samples were taken with a Nansen net with 200 µm mesh size in duplicate tows: one for analysis of species abundance and composition under binocular, while the other was preserved in ethanol for DNA extraction and metabarcoding. Metabarcoding was performed using mICOLintF-XT-jgHCO2198 primer pair which amplified a part of COI gene about 300 bp in length. *P. marinus* was detected in Mali Ston Bay in all seasons only by metabarcoding, but not by microscopy. Although this species was found in the surrounding area (Ploče harbour and Neretva river), this is its first detection in Mali Ston Bay. The present work confirms the high sensitivity of metabarcoding and its suitability for early detection of non-indigenous species. Its capacity to detect individuals with low relative abundances, as well as their early life stages (eggs or naupliar larvae), which are difficult to identify using conventional methods such as microscopy, can be a valuable tool for plankton monitoring.

9. Pasković, Nika

What is the origin of the rare dinoflagellates in the southern Adriatic Sea in 2023? // 5. hrvatski simpozij o invazivnim vrstama : zbornik sažetaka / 5th Croatian Symposium on Invasive Species : book of abstracts / Jelaska, Sven (ur.).

Zagreb: Hrvatsko ekološko društvo, 2023. str. 67-67

A few rare dinoflagellate species were found in samples collected in the open southern Adriatic Sea in spring - summer 2023 using Niskin bottles and plankton net. In this study, the samples were collected at four stations in the southern Adriatic Sea (P-1200, P-300, P-150, and P-100; the numbers indicate the maximum station depth). A total of 49 phytoplankton samples and four net samples were analyzed. All species have been previously recorded for the Adriatic and Mediterranean Sea. The rare species found are *Ceratoperidinium margalefii* A. R. Loeblich, *Tripos rotundatus* (Jørgensen) F. Gomez, *Pselodinium vaubanii* Sournia and *Amphisolenia globifera* F. Stein. These species can be considered as rare dinoflagellate species in the southern Adriatic Sea due to their percentage of occurrence (< 5%) in the analyzed samples. The highest abundance of *C. margalefii*, *P. vaubanii*, *A. globifera* were 20, 20 and 10 cells L⁻¹, respectively. One individual cell of the species *T. rotundatus* was found in all net samples. The origin of the dinoflagellates could be related to the different water masses entering the southern Adriatic Sea. The BiModal Oscillating System (BiOS) has an influence on the water masses entering the southern Adriatic Sea. According to the hydrographic data, the cyclonic phase of the BiOS was observed during the study period. The cyclonic phase of the BiOS means the intrusion of the saltier, denser, and nutrient-poor Levantine Intermediate Water, while the anticyclonic phase means the intrusion of the water from the Ionian Sea diluted by the Atlantic Water with lower salinity and density, but nutrient-rich. The BiOS also has an influence on the thermohaline properties and biodiversity in the southern Adriatic Sea.

10. Batistić, Mirna; Garić, Rade; Hure, Marijana

First occurrence of rare tropical hydromedusa *Paracystaeis octona* Bouillon, 1978 (Cnidaria: Anthoathecata) in the Adriatic Sea: A sign of climate changes? // Book of abstracts of 10th Workshop of Hydrozoan Society / Hosia, Aino; Martell, Luis; Soto-Angel, Joan (ur.).

Bergen: Norwegian University of Science and Technology, 2023. str. 6-6

Rare Indo-Pacific hydromedusa *Paracystaeis octona* (Bouillon, 1978) was collected for the first time at the open sea of southern Adriatic in December 2015. Since then, this species appears almost every year in the Adriatic Sea in similar periods. Some specimens were sequenced (COI, 16S rRNA, 18S rRNA) and the obtained sequences were searched against the GenBank database using the Blast tool but no sequence of any of species of this genus existed in GenBank. Nevertheless, this analysis confirms its phylogenetical position based on morphological characteristics that species belong to the family Cytaeidae. Relatively high number of specimens was occurred in a high salinity conditions (up to 39.3) in all years when they are registered. This indicates strong inflow of warmer and saltier East Mediterranean waters into Adriatic Sea. Accordingly, this species might be a Lessepsian migrant of Indo-Pacific origin. This could be a sign of ongoing climate changes and possible tropicalization of the Mediterranean Sea.

11. Garić, Rade ; Batistić, Mirna ; Dénes, Marcell ; Hure, Marijana ; Baričević, Ana ; Smodlaka-Tanković, Mirta
Investigations of gelatinous zooplankton in the Adriatic Sea in the last decade // Zbornik sažetaka 14. hrvatskog biološkog kongresa.
Zagreb: Hrvatsko biološko društvo, 2022. str. 198-199

Želatinozni zooplankton se pokazao kao dobar indikator termohalinih promjena u Jadranu. Brojnost i pojavnost stranih ili nedavno uspostavljenih želatinoznih vrsta su direktno povezani s promjenama u obrascima cirkulacije, a potencijalno i s razinom zagrijavanja Jadrana. U posljednjih deset godina u Jadranu su otkrivene dvije nove želatinozne planktonske vrste za znanost: *Brooksia lacromae* (Thaliacea, Tunicata) i *Aurelia pseudosolida* (Ulmaridae, Cnidaria). Indopacifička vrsta *Paracytaeis octona* (Cytaeididae, Cnidaria) je zabilježena od 2015. godine pa nadalje, dok su mediteranske vrste *Doliopsis rubescens* i *Pegea bicaudata* (Thaliacea, Tunicata) zabilježene u Jadranu prvi put 2016. godine. Sporadično su se bilježile i relativno rijetke vrste *Pyrosoma atlanticum* (Thaliacea, Tunicata), *Porpita porpita* (Porpitiidae, Cnidaria) i *Flaccisagitta hexaptera* (Chaetognatha). Naši podaci sugeriraju da se ubrzavaju promjene u sastavu zajednica želatinoznog planktona u Jadranskom moru što upućuje na potrebu provođenja sustavnih programa monitoringa na državnoj razini. Da bi se procijenila mogućnost korištenja metoda sekvenciranja sljedeće generacije u praćenju promjena u sastavu Jadranskog zooplanktona proveli smo istraživanje u Sjevernom i Južnom Jadranu u trajanju od godine dana. Naši preliminarni podaci pokazuju da se rijetke vrste zooplanktona i pelagički plaštenjaci ne bilježe konzistentno upotrebom fragmenta gena podjedinice I citokrom oksidaze (COI).

12. Dénes, Marcell ; Tóth, Gábor Endre ; Garić, Rade ; Hure, Marijana
Building the Adriatic Calanoid Database with the help of Oxford's MinION system // MetaZooGene Symposium - Presentation Abstracts.
2022. str. 5-5

Metabarcoding seems to be the future of biodiversity research. Simplifying time-consuming taxonomical methods, molecular approaches attract more and more ecology labs to join the new way of ecosystem evaluation each year. To achieve creditable results, reliable reference databases are needed. In response to this need we started building our own Adriatic multi-marker genetic database of calanoid copepods for our ongoing project. In this study we wanted to test the capabilities of the MinION sequencer for our purposes. All our samples were taken from our South Adriatic and North Adriatic coastal sampling stations through 2021. For the test, we used 42 specimens of calanoids with 4 markers (COI, 18S, 28S, ITS1-5.8S- ITS2) with fragment lengths ranging between 900-1300 BPs. We used previously acquired Sanger sequencing data to compare and verify our results. In the sequencing run 92.5% of the sequences were recovered with 99.95% final accuracy. 48% of the errors turned out to be indels with 1:2 deletion, insertion ratio. The most common errors were related to cytosine and guanine bases. The comparison of the two datasets also revealed, that sites with nucleotide repeats can cause ambiguous bases in the Sanger final product, while Nanopore resolved these sites at ease. The only drawback of the method seems to be its sensitivity for alien contamination, while using universal primers. In conclusion: with appropriate pre-and post-processing, MinION sequencing, with its ability to generate long barcodes is a viable alternative for Sanger sequencing in building genetic databases.

13. Batistić, Mirna ; Garić, Rade ; Hure, Marijana

Impact of the winter convective event on gelatinous zooplankton in the open southern Adriatic.
2022. str. 1-1. doi: 10.5194/egusphere-egu22-12875

The southern Adriatic is the deepest part of the Adriatic Sea (1242 m) and one of three sites of open-sea deep convection in the Mediterranean. By analyzing zooplankton samples taken in the open southern Adriatic in winter and spring/summer 2021 we investigated effect of winter vertical mixing on distribution of gelatinous zooplankton. During the convection time in winter, gelatinous zooplankton abundance was low and unusual vertical distribution for some species was occurred. In the spring-summer time an increase in gelatinous zooplankton abundance in upper and deeper layer was registered. This is probably related to the early spring phytoplankton bloom enhanced by nutrient input into euphotic zone due to winter mixing phase. As a consequence of this event, there is also availability of more food for deep- sea gelatinous organisms.

14. Batistić, Mirna ; Garić, Rade ; Hure, Marijana

Utjecaj zimskog vertikalnog miješanja na populaciju četinočeljista (chaetognatha) u južnom Jadranu // Zbornik sažetaka 14. hrvatskog biološkog kongresa.
Zagreb: Hrvatsko biološko društvo, 2022. str. 191-191

Južni Jadran je najdublji dio Jadranskog mora (1242 m) i jedno od tri mjesta dubokog vertikalnog miješanja u Sredozemnom moru. Analizom uzoraka zooplanktona uzetih na otvorenom moru južnog Jadrana u veljači i lipnju 2021., istražili smo utjecaj zimskog vertikalnog miješanja na rasprostranjenost i abundanciju četinočeljista od površine do 1200 m dubine. Krstarenje u veljači bilo je nakon epizoda jake bure i posljedično znatnih gubitaka topline na površini mora. Također, u intermedijarnom sloju zabilježen je ulaz slane istočnomediterranske struje (> 38, 9, LIW) koja uz navedene vremenske prilike stvara uvjete za vertikalno miješanje. Stoga je u veljači dubina miješanog sloja (DMS) bila do 600 m. U isto vrijeme zabilježena brojnost četinočeljista bila je niska i gotovo jednaka od površine do DMS- a. Također, zabilježena je neuobičajena vertikalna raspodjela epipelagičkih vrsta u relativno visokom broju od 200 do 600 m dubine. U lipnju, kada je uočena izražena toplinska stratifikacija zabilježeno je izrazito povećanje brojnosti četinočeljista u epipelagičkom sloju. To je vjerojatno povezano s proljetnim cvjetanjem fitoplanktona pojačanim unosom hranjivih tvari u eufotičnu zonu zbog zimske faze miješanja.

15. Hure, Marijana ; Dénes, Marcell ; Garić, Rade

The “SpaTeGen” project: metabarcoding calanoid copepods in the Adriatic Sea // Ocean Science Meeting 2022 - Abstracts.
2022. str. 1-1

Calanoid copepods are crucial components of mesozooplankton and pelagic ecosystems in general, showing high biological and ecological diversity. Due to diminishing number of taxonomical experts, investigations of zooplankton in recent times are concentrated onto small number of relatively large and easily identifiable species. Consequently, actual copepod diversity is often underestimated in oceanographic and ecological studies. Of the total of 185 Calanoida species in the Mediterranean, only 32 species (17%) have Mediterranean Sea-collected COI barcodes (WGIMT/MZGdb Atlas v2.01). The “SpaTeGen” project is aiming at overcoming these limitations by producing a database of genetic barcodes (18S- ITS1-5.8S-ITS2-28S and COI) of the Adriatic calanoid copepods. Here we present

preliminary data on the first project year, which includes COI and 18S-ITS1-5.8S-ITS2-28S sequences of more than 20 Adriatic calanoid species, sequence properties as well as potential metabarcoding target sites. The aim is to produce at least 40 barcodes per project year, ending in almost all Adriatic calanoid copepods barcoded by 2026. In order to collect Adriatic calanoid copepods the sampling will cover wide spatial area, including characteristic coastal eastern Adriatic systems as well as its shallow northern and deep southern part up to 1200 m depth. Keywords: biodiversity, DNA barcode, Mediterranean Sea, mesozooplankton

16. Hure, Marijana ; Batistić, Mirna ; Garić, Rade

VERTIKALNA DISTRIBUCIJA BROJNOSTI I RAZNOLIKOSTI KOPEPODA POD RAZLIČITIM UVJETIMA OKOLIŠA U JUŽNOM JADRANU // ZBORNİK SAŽETAKA 14. HRVATSKOG BIOLOŠKOG KONGRESA. Zagreb: Hrvatsko biološko društvo, 2022. str. 199-199

Vertikalna migracija je rašireno ponašanje mnogih svojti zooplanktona kroz koje organizmi aktivno sudjeluju u transportu tvari i energije u morskom okolišu. Cilj ovog istraživanja bio je razjasniti promjene u vertikalnoj distribuciji zajednica kopepoda pod dva različita okolišna uvjeta: ljetna stratifikacija (lipanj 2020) i događaj duboke zimske konvekcije (veljača 2021). Uzorci su uzeti tijekom ciklusa od 24 sata na jednoj postaji u južnom Jadranu od površine do 1200 m (u osam dubinskih slojeva). U lipnju je najveća brojnost kopepoda zabilježena u površinskom sloju tijekom ranog jutra, dok je tijekom intenzivnog dnevnog svjetla najveći dio populacije kopepoda bio koncentriran na sloju od 200-300 m. U veljači su maksimalne vrijednosti gustoće bile u površinskom sloju (0-50 m) tijekom cijelog ciklusa od 24 sata. Ukupno je identificirano 90 svojti kopepoda. Vertikalni obrasci raznolikosti pokazali su veće vrijednosti u srednjim slojevima (400-600 m) tijekom podneva u oba godišnja doba. Izračunata srednja dubina pojavljivanja (WMD) za većinu istraživanih vrsta kalanoida bila je dublja u veljači nego u lipnju, što ukazuje na njihovo zadržavanje u dubljim slojevima tijekom razdoblja miješanja, a time i smanjuje rizik gubitka putem vizualnih grabežljivaca. Naši podaci naglašavaju važnu ulogu gradijenata okoliša u vertikalnom strukturiranju zajednica kopepoda u obje vremenske skale (dnevne i sezonske), u dinamičnom oligotrofnom pelagičkom sustavu.

17. Hure, Marijana ; Batistić, Mirna ; Garić, Rade

Diel vertical distribution of copepod abundances and diversity in the open South Adriatic Sea (NE Mediterranean) // Book of abstracts 55th European Marine Biology Symposium. Gdanjsk: EMBS, 2022. str. 62-62

Copepod community structure was investigated on a short time sampling scale during a 24-h cycle at one fixed station in the open Adriatic Sea in June 2020 and February 2021 (from 0 to 300 m) using Nansen opening-closing net (250 μ m mesh size). In June, the upper oceanic waters were characterized by vertical gradients of environmental factors while in February oceanic waters showed relatively homogeneous physicochemical conditions. During the winter vertical mixing, the bulk of the copepod population remained in the epipelagic zone (0- 100 m) over the entire 24-h cycle, with calanoids remained the dominant group. An increasing trend of copepod standing stocks from midnight to early morning in the surface layer found in June is in agreement with previous records of copepod day- night variations in the Mediterranean Sea. Average Shannon-Wiener diversity index was lower in July (2.21 ± 0.27) than in February (2.54 ± 0.18) with the most pronounced differences in a 200-300 m layer,

during the whole sampling period. Day-night differences in diversity and number of taxa of the epipelagic area were more pronounced in June, confirming the higher intensity of diel vertical migration in summer as well as deficiency of calanoids during the most intensive daylight. Although the epipelagic community was composed of numerous weak diel vertical migration species, for the majority of investigated copepod taxa migration patterns differed between the seasons suggesting significant influence of environmental conditions on their vertical positioning.

18. Garić, Rade ; Dénes, Marcell ; Hure, Marijana ; Batistić, Mirna
Barcoding of thaliacea in the Adriatic // Ocean Science Meeting 2022 - Abstracts.
2022, 7946, 1

The coverage of Thaliacea species has been relatively good regarding 18S. The 18S gene is highly conserved, therefore of limited use in delimiting closely related species and unsuitable for population-level studies. This is demonstrated by the fact that *Thalia democratica* and *Thalia longicauda*, which are morphologically quite distinct, possess identical 18S sequences. COI has been traditionally the most common barcode gene, because of its ability to differentiate species and populations and relative ease of amplification across taxa, but thaliacean COI sequences are scarce, so much so that COI barcodes of only five Thaliacea species are available. The general absence of available thaliacean sequences is likely due to a combination of factors: lack of taxonomists as well as inability of the commonest COI primers (namely, LCO1490 and HCO2198) to reliably amplify COI gene fragments across thaliacean taxa. Within the framework of SpaTeGen project, in an effort to produce COI sequences of Adriatic thaliaceans, we combined multiple primer pairs and produced doliolid and salpid COI sequences of more than 800 bps, encompassing HCO2198 binding site. Our preliminary results show that pyrosomatid and doliolid sequences are 6 aminoacids longer than salpid sequences, in the first 300 aminoacid stretch. Preliminary phylogenetic analysis based on COI sequences place salpids as a sister clade to pyrosomatids and doliolids. This is in contrast to published phylogeny based on 18S, which place pyrosomatids as the most ancestral group from which doliolids and salpids branch off in succession.

19. Dénes, Marcell ; Hure, Marijana ; Garić, Rade
System inconsistencies: investigating the phylogeny of calanoid copepods // Zbornik sažetaka
14. hrvatskog biološkog kongresa.
Zagreb: Hrvatsko biološko društvo, 2022. str. 195-195

Calanoida, as part of the most abundant taxa of the meso-zooplankton community, plays and undeniably important role in the marine food-chain and nutrient circulation. The number of species are usually underestimated, mostly due to complicated identification characters and the lack of taxonomic experts. With the combination of molecular methods and standard approaches, it is getting easier to uncover the hidden diversity within the group. The available genetic databases show that the 185 Calanoid species of the Mediterranean are still underrepresented (16%) even in the most commonly used marker (COI). Our project "SpaTeGen" is focused on building a database of genetic barcodes, covering the blank spots using a handful of markers (COI, 18S, 5.8S, ITS2, 28S), to help future species identification and biodiversity monitoring of the Adriatic region. Our knowledge must also be broadened for a better understanding of their evolutionary connections. Our preliminary results show that phylogeny based on genetic markers does not seem to support currently accepted systematics and that

three calanoid families (Aetideidae, Euchaetidae and Clausocalanidae) might be polyphyletic.

20. Garić, Rade ; Batistić, Mirna ; Dénes, Marcell

New species of Doliolida (Tunicata, Thaliacea) from the Adriatic Sea // MetaZooGene Symposium - Presentation Abstracts.

2022. str. 7-7

New species of Doliolida is found in the South Adriatic in autumn 2021. Blastozooids of this species are similar to *Dolioletta gegenbauri* in endostyl position (from MII 1/2 to MIV 1/3), overall body consistency (both species are fragile and easily damaged in net tows), position and length of the testis and ovary and the position of the branchial bar. The main difference in *Dolioletta* sp. nov. and *Dolioletta gegenbauri* is in that intestine does not form a tight coil and after forming a right loop as in *Doliolum nationalis*, it sharply turns left so that anal aperture is facing left body wall. The other difference between these two species is in that the ganglion is positioned between MIII and MIV, but closer to MIV in *Dolioletta* sp. nov., while in *Dolioletta gegenbauri* it is positioned closer to MIII. COI phylogenetic analysis showed that *Dolioletta* sp. nov. forms a clade with *Dolioletta gegenbauri* (100% bootstrap support), while the COI sequence difference between these two species is 16.8%. The finding of a new doliolid species in the Adriatic Sea continues the worrisome trend in recent decades of new gelatinous species being described every couple of years in this well investigated area. The geographic origin of this species is still unknown.

21. Dénes, Marcell ; Tóth, Gábor Endre ; Garić, Rade ; Hure, Marijana

Building the Adriatic Calanoid Database with the help of Oxford's MinION system // MetaZooGene Symposium - Presentation Abstracts.

2022. str. 5-5

Metabarcoding seems to be the future of biodiversity research. Simplifying time-consuming taxonomical methods, molecular approaches attract more and more ecology labs to join the new way of ecosystem evaluation each year. To achieve creditable results, reliable reference databases are needed. In response to this need we started building our own Adriatic multi-marker genetic database of calanoid copepods for our ongoing project. In this study we wanted to test the capabilities of the MinION sequencer for our purposes. All our samples were taken from our South Adriatic and North Adriatic coastal sampling stations through 2021. For the test, we used 42 specimens of calanoids with 4 markers (COI, 18S, 28S, ITS1-5.8S- ITS2) with fragment lengths ranging between 900-1300 BPs. We used previously acquired Sanger sequencing data to compare and verify our results. In the sequencing run 92.5% of the sequences were recovered with 99.95% final accuracy. 48% of the errors turned out to be indels with 1:2 deletion, insertion ratio. The most common errors were related to cytosine and guanine bases. The comparison of the two datasets also revealed, that sites with nucleotide repeats can cause ambiguous bases in the Sanger final product, while Nanopore resolved these sites at ease. The only drawback of the method seems to be its sensitivity for alien contamination, while using universal primers. In conclusion: with appropriate pre- and post-processing, MinION sequencing, with its ability to generate long barcodes is a viable alternative for Sanger sequencing in building genetic databases.

Prilog sa skupa (neobjavljen)

Neobjavljeni prilog sa skupa

22. Garić, Rade; Batistić, Mirna; Hure, Marijana; Baričević, Ana; Smodlaka Tanković, Mirta; Licandro, Priscilla; Dénes, Marcell
Barcoding of non-indigenous gelatinous zooplankton species in the Adriatic // 57th European Marine Biology Symposium Naples
Napulj, Italija, 16.09.2024-20.09.2024

Over the past three decades, a persistent influx of non-indigenous gelatinous zooplankton species into the Adriatic Sea has been observed. Among these species, some have firmly established themselves as permanent members of the Adriatic zooplankton community, while others appear periodically, their presence contingent on the prevailing water masses entering the Adriatic. Furthermore, a number of previously undocumented species have been recently discovered in the Adriatic, and their long-term impact remains uncertain. With the advent of genetic techniques there has been increasing need to genetically profile these species, aiming to unveil their origins, whether from the Atlantic or the Indopacific, and to assess the utility of metabarcoding as a monitoring method for detecting non-indigenous gelatinous zooplankton species. We barcoded 14 species non-indigenous species in the Adriatic: *Thalia orientalis*, *Brooksia lacromae*, *Dolioletta advena*, *Doliopsis rubescens*, *Pegea bicaudata*, *Pyrosoma atlanticum*, *Aurelia pseudosolida*, *Paracytaeis octona*, *Muggiaea atlantica*, *Porpita porpita*, *Charistephane fugiens*, *Pontodora pelagica*, *Pelagobia longicirrata*, *Fritillaria helenae*, and *Oxygyrus inflatus*. Over a 12-month period, samples for metabarcoding were collected from both the South and North Adriatic. In the Mediterranean, non-indigenous species arrive through the Suez Canal or Gibraltar. Identifying their origins informs us which entry point has a greater impact on the Adriatic Sea, aiding in predicting future changes. However, genetic analysis alone in this case was mostly unable to pinpoint their origin due to limited genetic reference data. A multi-marker approach in metabarcoding has shown the best results in detecting non-indigenous species within the Adriatic ecosystem.

23. Hure, Marijana; Batistić, Mirna; Garić, Rade
Diel and seasonal vertical migration of *Calanus helgolandicus* and *Neocalanus gracilis* in the deep southern Adriatic (NE Mediterranean) // ICES-PICES 7th International Zooplankton Production Symposium
Hobart, Australija, 17.03.2024-22.03.2024

Vertical habitat selection of populations of *Calanus helgolandicus* and *Neocalanus gracilis* (CV and adults) from the open oligotrophic waters of the Adriatic Sea (~1250 m) at different temporal scales is described and compared. A total of 120 day-night samples were collected on a seasonal basis (from June 2020 to May 2023) using an opening-closing Nansen net at eight sampling depths. Vertical behavior differed among species: seasonal rather than diel vertical migration dominated for *C. helgolandicus*, while *N. gracilis* showed a classic diel vertical migration pattern throughout the year. *N. gracilis* is a typical mesopelagic species, with a core population at about 220 m depth. In September, the maximum

of 0.65 ind./m³ was measured in the 200-300 m layer in daily samples. In contrast, *C. helgolandicus* has two separate populations during most of the year: one at the surface, concentrated in the upper 100 m depth, and the deeper-living one below 400 m depth. The seasonal numerical peak of abundance (>4 ind./m³) was recorded in May (800-1200 m layer). During the warmest period, the surface population of *C. helgolandicus* disappears, indicating oversummering, whereas during winter mixing it was found throughout the water column. In contrast to the typical diel vertical migration of *N. gracilis*, *C. helgolandicus* rose only at night during winter. The differential responses of these species to environmental factors provide a foundation for future research that will expand our knowledge of biological interactions, vertical transport of organic carbon, and copepod adaptations to future climate change.

24. Njire, Jakica; Bojanić, Natalia; Lučić, Davor

Microzooplankton (tintinnid ciliates) diversity: deep-sea vs. coastal community structure in the southern Adriatic Sea // 56th European Marine Biology Symposium
Reykjavík, Island, 04.09.2023-08.09.2023

Tintinnid ciliates are ideal organisms for studying changes in diversity caused the rapidly hydrographical and varying meteo-oceanographic conditions. Regarding this, two different ecological regions in the Adriatic sea were select: the deep central part of the south Adriatic and river Neretva shallow estuary in the south-eastern Adriatic coast. The southern Adriatic is a unique area with the deepest and the widest part of the basin. During winter, changes in hydrodynamics influenced by winds and regular water exchange with the adjacent Ionian Sea and the northern part of the Adriatic Sea largely impact on the occurrence of diversity, abundance and distribution of tintinnid ciliates. The results of our research in south Adriatic showed important diversity: 63 species were recorded, 62 in December and 41 in April. According to spatial distributions, we found 40 surface species (including neritic-estuarine), 17 subsurface species, five mesopelagic species, and one deep-sea species. The highest species richness was recorded in the surface and subsurface layers in December. In April, a shift of population toward greater depths and modification of deep sea community structure with decreased diversity was recorded as a consequence of the winter convection-driven sinking of tintinnids. We also recorded some species known as immigrants from either the Eastern Mediterranean or the Ionian Sea. The Neretva River estuary is a salt-coast type, classified as “transitional waters”: the main characteristic is the occurrence of pronounced vertical stratification of the water column in terms of salinity, nutrient concentration, oxygen content, and plankton community composition. The results of our estuary’s research showed unexpectedly high tintinnid diversity: 47 species were recorded, 44 at estuary station and 38 at upstream station. The number of oceanic species was almost equaled to the neritic-estuarine species indicating strong intrusion of a seawater wedge into the riverbed itself. Significantly higher population densities at the upstream station indicate favourable environmental conditions. The lowest diversity and abundance reported in the surface layer, indicating lack of intense vertical mixing of the layers. This research has confirmed the highest species richness values were in the southern Adriatic Sea during the winter. Contrary to our expectation, in the Neretva river estuary, high numbers of tintinnid species and high diversity was detected in summer months.

25. Garić, Rade ; Denes, Marcell ; Hure, Marijana ; Baričević, Ana ; Smodlaka Tanković, Mirta ; Batistić, Mirna
Comparison between COI metabarcoding and microscopy for zooplankton monitoring of the Adriatic biodiversity // ICES Annual Science Conference 2023
Bilbao, Španjolska, 11.09.2023-14.09.2023

Most metabarcoding studies focus on limited temporal sampling, which can result in missing seasonally present species. To assess the efficacy of COI metabarcoding as a monitoring method and compare its results to classical methods of formalin sample analysis under the loupe, we sampled a 100 m deep station in the South Adriatic and a 20 m deep station in the North Adriatic in duplicate samples using a 200- μ m plankton net, from bottom to surface, monthly from March 2021 to February 2022. We used the mICOLintF-jgHCO2198 primer pair and an MZG COI referent database enriched with zooplankton sequences produced from our own barcoding efforts for zooplankton community assessment using COI metabarcoding, including most common zooplankton taxa not yet present in GenBank or BOLD, such as *Muggiaea kochii*. Our results indicate that the calanoid copepod composition obtained by metabarcoding largely matched those obtained by microscopy, but metabarcoding produced a larger number of species for some genera, such as *Paracalanus*. The metabarcoding approach also recovered the diversity of chaetognaths, pteropods, and heteropods well for abundant taxa, but it largely failed to detect rare taxa and pelagic tunicates. It did not produce any salpid sequences and only recorded two doliolid species, one of which is a newly described species, *Dolioletta advena*. For Appendicularia, metabarcoding only detected *Oikopleura longicauda*, while for Cnidaria, COI metabarcoding was mostly successful in reproducing community composition, but it consistently produced large numbers of *Nectadamas diomedea* sequences, a species not previously recorded in the Mediterranean according to WoRMS or in our formalin samples. COI metabarcoding was largely able to reproduce the temporal and spatial variability of zooplankton, and it is a good substitute for classical methods for many taxa. However, given the limitations of the metabarcoding approach, we recommend its use in combination with classical methods to obtain a more comprehensive understanding of the biodiversity of zooplankton in the Adriatic Sea.

26. Baricevic, Ana; Smodlaka Tankovic, Mirta; Hure, Marijana; Dénes; Garic, Rade; Batistic; Mirna,
Comparison of the three metabarcoding genes (18S, 28S and COI) for the Adriatic Sea
zooplankton biodiversity monitoring // ICES Annual Science Conference 2023
Bilbao, Španjolska, 11.09.2023-14.09.2023

The monitoring of zooplankton in the Adriatic Sea has long been relied on traditional methods of biodiversity description using stereomicroscopy, but the development and more common use of metabarcoding in biodiversity studies significantly improved community completeness and high-throughput sample processing in zooplankton monitoring. The choice of metabarcoding genes represents an important step for the successful amplification of taxonomically diverse zooplankton samples. During a one year period (March 2021 – February 2022), monthly zooplankton sampling was carried out in the northern and southern Adriatic Sea and net samples were processed for biodiversity assessments using metabarcoding. To achieve successful detection of diverse zooplankton taxa, three barcodes (18S, 28S and COI) were used. Detailed community compositions of the three barcodes were compared to evaluate taxa coverage and potential of each barcode in targeting organisms of specific interest for the

monitoring studies (e.g. non-indigenous species). The highest number of different invertebrate taxa (267) was identified with 18S while 28S and COI identified 144 and 201 invertebrate taxa, respectively. Holoplankton and meroplankton representatives of the 9 major invertebrate phyla (Annelida, Arthropoda, Bryozoa, Chaetognatha, Chordata, Cnidaria, Ctenophora, Echinodermata and Mollusca) were identified with all three barcodes, while 18S and 28S enabled identification of the phylum Nemertea representatives as well. Phylum Arthropoda dominated all three metabarcoding datasets (18S, 28S and COI) in reads number with majority of all Arthropoda taxa classified to Copepoda. The 18S and COI datasets showed a much higher richness of copepod taxa (50 and 66 taxa, respectively) compared to 28S (9 taxa). Only 15% of the identified copepod taxa were shared between 18S and COI, while others were barcode specific. Genus *Paracalanus* was the most abundant copepod taxa in datasets of all three barcodes. 28S barcode was characterised with lower taxa richness for all the detected phyla compared to the other two barcodes. Overall, multigene metabarcoding confirmed significant differences between zooplankton communities of the northern and southern Adriatic station, and temporal patterns for each region were also present. Our results confirm that robustness and extensive approach of metabarcoding have the potential to complement traditional zooplankton identification methodologies in monitoring.